ASPECTS OF THE LIFE HISTORY OF *MUSPICEA BORRELI* (NEMATODA: MUSPICEIDAE), PARASITE OF THE HOUSE MOUSE (*MUS DOMESTICUS*) IN AUSTRALIA

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Summary:

Prevalence of *Muspicea borreli* (Nematoda) infection in wild populations of *Mus domesticus* in forests in southeastern New South Wales and in rural Canberra, Australia was variable, relatively low and the parasite occurred predominantly in male mice. Experimental infection of BALB/c mice occurred only via subcutaneous inoculation but was achieved using *i*) adults containing embryonating eggs, *ii*) adults containing active larvae and *iii*) active larvae dissected from the uterus of female worms. Experimental infection was not established using adults containing unembryonated eggs and was not established via intraperitoneal, percutaneous nor oral routes. Evidence indicates that larvae develop to the infective stage in the uterus of the adult worm, suggests that an obligate developmental phase on the host skin does not occur and that autoinfection is possible. Experimental infection predominated in males; females rarely became infected. When male BALB/c mice were inoculated subcutaneously with *M. borreli*, immediately paired with an uninoculated female and permitted to breed for 90 days, infection was found in male and female offspring only of the second and subsequent litters or in the breeding female partner. Transmission to the young occurred within 21 days of birth and fifth-stage *M. borreli* were found in offspring of the second and subsequent litters only after 35 or more days. However, when a male was inoculated but mating delayed for 23 days, infection was found in progeny of the first and second litters. The life cycle is direct and the prepatent period in BALB/c mice is estimated at 50-60 days. The precise mode of transmission of the parasite in breeding pairs of mice was not determined but larvae remained active for approximately an hour in balanced saline solutions (pH = 7.2) and in human saliva but died under conditions emulating free-living (tap water pH = 7.1) and stomach (pepsin solution pH = 2) environments. Transmission was not effected by transplacental, transmammary nor transseminal routes. Consequently, it is difficult not to conclude that transmission may occur via penetration of skin or mucous membranes, and allogrooming behaviour may be particularly important in this regard.

KEY WORDS : *Muspicea borreli*, Nematoda, Muspiceidae, life history, distribution in wild populations, *Mus domesticus*, Australia.

Résumé : DONNÉES SUR LE CYCLE BIOLOGIQUE DE *MUSPICEA BORRELI* (NEMATODA: MUSPICEIDAE), PARASITE DE LA SOURIS DOMESTIQUE (*MUS DOMESTICUS*) EN AUSTRALIE

Chez les populations sauvages de Mus domesticus *des forêts du sud-est du New South Wales et des zones rurales de Canberra, la prévalence de* Muspicea borreli *est variable, généralement faible, et elle prédomine chez les mâles. Les infestations expérimentales des souris BALB/c n'ont réussi que par voie sous-cutanée, en inoculant des adultes contenant des œufs embryonnés ou contenant des larves actives, ou en inoculant des larves actives extraites des utérus. Les infestations ont échoué en sous cutané quand les adultes avaient des œufs non embryonnés, ainsi qu'après inoculation intrapéritonéale, ou dépôt des larves sur la peau, et* per os. *Les larves atteignent le stade infestant dans l'utérus des adultes, ce qui suggère qu'il n'y a pas de phase libre sur la peau de l'hôte et que l'autoinfestation est possible. Dans les conditions expérimentales, les BALB/c femelles s'infectent beacoup plus rarement que les mâles. Quand les BALB/c mâles sont inoculées en sous-cutané et appariées immédiatement avec des femelles non inoculées, et ces couples maintenus pendant 90 jours, l'infection s'observe chez les descendants des deux sexes, à partir de la seconde portée, et chez la mère; la transmission au jeune a lieu au cours des 21 premiers jours de sa vie; le stade adulte s'observe à partir de 35 jours chez la seconde portée et les suivantes. Quand un mâle est apparié 23 jours après avoir été déjà inoculé, la première portée elle-même est déjà contaminée. Le cycle biologique est direct et la période prépatente est estimée à 50-60 jours chez la BALB/c. Le mécanisme de la transmission chez les couples reproducteurs n'est pas totalement élucidé mais on a constaté que les larves restent actives environ une heure dans une solution salée tamponnée à pH 7,2 ainsi que dans la salive humaine, tandis qu'elles meurent dans l'eau du robinet (pH 7,1) et dans une solution pepsique (pH 2) reproduisant le milieu gastrique. La transmission n'a pas lieu par voie transplacentaire, transmammaire et transéminale, en conséquence il est difficile de ne pas conclure que la transmission doit se faire par pénétration de la peau ou des muqueuses, le comportement de léchage réciproque (grooming) apparaissant particulièrement important à cet égard.*

MOTS CLÉS : Muspicea borreli, *Nematoda, Muspiceidae, cycle évolutif, distribution dans les populations sauvages,* Mus domesticus, *Australie.*

INTRODUCTION

Muspicea borreli was discovered in 1911 by Professor A. Borrel, Director of the Bacteriological Institute of Strasbourg, while investigating the role of parasites in the genesis of cancer. The nematode was described by Sambon (1925) on the basis of seven female specimens from the subcutaneous connective tissue and inguinal glands of the common mouse, *Mus musculus*, in Strasbourg. Sambon suggested that *M. borreli* had probably been seen by Redi in 1684 in the inguinal glands of the mouse. The family Muspiceidae was erected by Brumpt (1930) for *M. borreli*, who also reported transmission of *M. borreli* by unknown means in colonies of laboratory mice. No new records of this parasite were reported any-

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where in the world until studies of the parasites of wild mice, *Mus domesticus*, in Australia (Singleton, 1985; Singleton & Redhead, 1990). However, it was found in laboratory mice in Paris in 1979 and at Yaoundé in breeding colonies at the Institut de Recherches et Développement (Bain, *pers. comm.).*

M. borreli is a protandrous hermaphrodite, spermatogenesis occurring in the wall of the genital pouch and terminating when the distal cells of the genital cord commence division for ovogenesis. The vulva is atrophied and eggs hatch inside a uterine pouch, develop simultaneously to third-stage larvae and burst from the head region killing the adult (semelparity), a developmental feature known as *endotokia matricida* (Seurat, 1914) which offers an efficient mechanism for auto-reinfection of the host.

The life cycles of muspiceoid genera remain speculative but cannibalism (i.e. necrophagy), cutaneous penetration, lactation and grooming have been proposed as routes of infection (Sambon, 1925; Brumpt, 1930; Roman, 1965; Bain & Chabaud, 1968, 1979; Chabaud & Bain 1974; Bain & Nikander, 1982; Spratt & Speare, 1982; Anderson 1984). Adamson (1986) suggested that these parasites have monoxenous life cycles and have evolved in their hosts directly from soil-dwelling ancestors because no intermediate hosts have been recognised. Subsequently however, larvae of a presumed muspiceid were reported from *Ixodes dammini* (Acari: Ixodidae) (Beaver & Burgdorfer, 1984, 1987) and infective larvae of a presumed new species of Robertdollfusidae were reported from the gut of *Simulium damnosum* (Diptera: Simuliidae) in Cameroon (Bain & Renz, 1993). In addition, Spratt & Nicholas (2002) reported larval *Durikainema* sp. from the abdomen of *Culicoides victoriae* (Diptera: Ceratopogonidae) near Atherton, Queensland where the tree kangaroo, *Dendrolagus lumholtzi,* is known to harbour *Durikainema macropi.*

The most primitive muspiceoid life cycles are thought to involve little tissue migration (Bain & Chabaud, 1979). Larvae are believed to penetrate the skin, develop in the subcutaneous tissues and transmission stages are thought to leave through a skin lesion and seek a new host (Adamson, 1986). A tissue migration would become necessary when percutaneous transmission was replaced by oral transmission as may occur in *Muspicea* and *Lappnema* (Brumpt, 1930; Bain & Nikander, 1982). Presumably, parasites of the deeper tissues eg. *Durikainema* spp. and *Haycocknema perplexum* are derived from these tissue migrating forms (Adamson, 1986).

This paper reports on the distribution of *Muspicea borreli* in wild populations of house mice (*Mus domesticus*) in Australia. It also provides data on experimental infection of BALB/c mice and monitoring of natural transmission of *M. borreli* from these mice to their offspring under laboratory conditions.

MATERIALS AND METHODS

Distribution in wild mice

Wild *Mus domesticus* were live-trapped using Longworth or Elliott traps with oats, wheat grain or a mixture of peanut butter and rolled oats as bait. Trapping occurred at Nadgee State Forest (149° 51' E, 37° 18' S) and Nature Reserve (149° 52' E, 37° 27' S) and at Timbillica (149° 55' E, 37° 36' S) and Buckenboura (150° 02' E, 35° 42' S) State Forests in southeastern New South Wales between 1981 and 1991. Trapping also was conducted at Canberra in the Australian Capital Territory (149° 08' E, 35° 09' S) from February through to August, 1988.

The following information was recorded for each mouse trapped: location, sex, reproductive condition and weight. Mice were classed as juvenile (< 12 gm), subadult (12-15 gm) or adult (> 16 gm) (Singleton, 1985). Prevalence of infection in relation to host age, reproductive condition and sex were compared using Chi^2 analysis. Unpaired two-tailed t-tests or ANOVA were used to compare mean nematode intensity in the above categories. A Chi^2 goodness of fit test was used to eliminate the degree of randomness in the frequency distribution of *M. borreli* in wild mice. Quantitative parasitological terms used in this study follow Bush *et al.* (1997).

Mice were killed by cervical dislocation, carcasses were skinned and carcass and skin were treated by one of two different methods. In the first method skin and carcass were washed in Hank's balanced salt solution (HBSS) (pH = 7.2) in a Petri dish, gently scraped with a razor blade, then rewashed. The skin was cut into five pieces, rinsed into the top of a Baermann apparatus containing HBSS, covered with a Petri dish and held at 37° C for three hours, agitating the immersed skin every 15 minutes with a glass stirrer or forceps. At 15-30 minute intervals fluid was tapped off into a test tube, allowed to stand, the supernatant removed with a pipette and the bottom 2 cm examined in a Petri dish under a dissecting microscope. In mice known to be infected in the subcutaneous region, the liver, spleen, lymph nodes, lungs, heart, brain, mammary glands, genitalia, tongue and lips were teased apart in HBSS under a dissecting microscope and examined for nematodes. The tissues subsequently were immersed in HBSS in a test tube, allowed to stand at 37° C for an hour and the fluid then re-examined for nematodes under a dissecting microscope. In the second method, skin and carcase were placed in separate Petri dishes containing 0.9 % saline. The skin was then teased apart using jeweller's forceps, allowed to stand at room temperature for 15-30 minutes then examined for nematodes under a dissecting microscope. The intact carcass was allowed to stand at room temperature for

15-30 minutes and then examined for nematodes under a dissecting microscope using both incident and transmitted light. Subsequently all tissues and organs listed above were dissected and examined for parasites.

EXPERIMENTAL INFECTIONS

Experiments were conducted in uninfected male and female BALB/c mice to determine which stages of development of *M. borreli* *i*) adult nematodes with unembryonated eggs (Au), *ii*) adults containing embryonating eggs (Ae), *iii*) adults containing larvae (Al) and *iv*) active larvae approximately 0.5 mm in length dissected from the uterus of female worms (Lu) were infective to mice (Table I). In these experiments we sought also to determine the most appropriate method for infecting mice. Consequently, the four stages listed above were administered to male and female mice by either the subcutaneous or intraperitoneal route (Table I). In addition, active larvae dissected from the uterus of females were administered to male and female mice by oral and percutaneous (i.e. placing larvae on skin) routes (Table I).

Virgin male and female mice were lightly anaesthetised with ether vapour before experimental infection. *M. borreli* recovered initially from wild-caught *M. domesticus* and subsequently from BALB/c mice were washed in saline and 0.5-1.0 ml of saline containing worms was administered subcutaneously or intraperitoneally using a 19 gauge needle. For percutaneous infection, uterine larvae in 0.2 ml saline were placed on the abdominal skin of 5-10 day old mice and lightly covered to keep larvae moist. For oral infection, 0.5 ml of saline containing nematodes was administered *per os*. Experimental doses ranged from one-five juvenile or adult stages and from 10-60 larval stages. Mice were examined at intervals ranging from five to 90 days after infection (DAI). Mice were maintained in animal facilities at 22 ± 2° C on dry sawdust, with food and water *ad libitum*. Cages and sawdust were changed each week and cages autoclaved.

When the results of these experiments were available, we sought to determine if direct transmission occurred from infected male to uninfected female (n = 5) or from infected female to uninfected male (n = 3). Male or female mice were inoculated subcutaneously and then housed together in pairs from the day of inoculation, 25 days after inoculation and 102 days after inoculation for periods ranging from six to 149 days.

Additional experiments were conducted to determine if the mode of transmission was from *i*) male to female (trans-seminal), *ii*) female to male, *iii*) between parents and offspring (trans-seminal, trans-placental, trans-mammary). Sexually naive male and female BALB/c mice were inoculated subcutaneously with larvae dissected from the uterus of female *M. borreli* then placed in breeding pairs with an uninfected mouse and permitted to breed for 90 days with all litters being weaned at 19-25 days (X = 21 days) and subsequently housed by sex and separately from parents. Breeding pairs were checked daily for births and deaths. Mice from each litter were examined at intervals ranging from 35 to 61 days of age. Parents were examined during and at the termination of the breeding experiments. In one instance a male was inoculated subcutaneously with adult *M. borreli* but not mated with an uninoculated female until 23 days later. The progeny of the first litter of this mated pair were examined at 37 (n = 2) and 54 (n = 6) days of age.

Additionally, *M. borreli* were assessed *in vitro* to determine the tolerance of larval and adult stages to different physical, chemical and environmental conditions. Survival was assessed after placing nematodes in *i*) Hank's balanced saline solution (pH = 7.2), *ii*) tap water (pH = 7.1), *iii*) a 1 % pepsin solution made up with saline (pH = 2) and *iv*) human saliva.

All research work was conducted under approval from both the CSIRO and the Australian National University Animal Ethics Experimentation Committees.

RESULTS

DISTRIBUTION IN WILD MICE

M. borreli was found exclusively in the subcutaneous tissues of wild *Mus domesticus*. All known stages in the muspiceid life cycle were recovered from this site but the frequency of

Location	No. examined		No. infected		Prevalence %		Intensity*	
	Male	Female	Male	Female	Male	Female	Male	Female
Canberra	78	64	16	6	20.5	9.4	1-18 (2.9)	1-2 (1.2)
Nadgee SF & NR	57	68	0	0	0	0	0	0
Timbillica SF	54	29	2	0	3.7	0	1-11 (6.0)	0
Buckenboura SF	8	7	4	0	50.0	0	1-6 (3.8)	0
Totals	197	168	22	6	11.2	3.6	–	–

* Mean intensity in parentheses.

Table I. – Prevalence and intensity of *Muspicea borreli* infection in wild *Mus domesticus* in southeastern Australia.

recovery differed between different life cycle stages. In Canberra, prevalence of infection was significantly higher in male than female mice ($p < 0.025$) and increased significantly with weight in male mice only ($p < 0.01$). Prevalence of infection in reproductive males was significantly greater than in non- reproductive males ($p < 0.005$). Frequency distribution of worm burdens per host was highly skewed in male mice and did not fit a random distribution ($X^2 = 20$, d.f. = 2, $p < 0.001$). The majority of infected mice had one-two nematodes, however some mice had up to 18 nematodes. Mean intensity of infection was greater in male than female mice (Table I). One infected female was imperforate, five were perforate and of the latter, two were lactating.

In Timbillica State Forest, *M. borreli* was found in only two adult male mice and intensity ranged from 1-11 (Table I). No infected mice were found in Nadgee State Forest and Nature Reserve. In Buckenboura State Forest, *M. borreli* was found exclusively in adult male mice, prevalence of infection was high and intensity of infection ranged from one-six (Table I).

Experimental infections

Experimental infection of laboratory mice with *M. borreli* was established by subcutaneous inoculation of *i)* adult nematodes containing eggs, *ii)* adults containing active larvae and *iii)* active larvae dissected from the uterus of female nematodes. Experimental infection was not established by application of larvae to skin or by oral or intraperitoneal routes (Table II). Experimental infections were established primarily in male mice (18 of 24); infection was established in only one of 16 females.

Transmission of *M. borreli* occurred from infected male mice to females on all three occasions (Table III) when they were housed as pairs from the day of inoculation until 137-149 DAI, when one pair were together from 102-145 DAI but not when one pair were together only from 25-31 DAI. All males were infected at post mortem examination. Transmission of *M. borreli* occurred from infected female mice to males on only one of three occasions (Table III) when one pair were housed together from the day of inoculation until 112 DAI but not when two pairs were housed together until 99 and 137 DAI. However, only one of the three females was infected at post mortem examination.

Litters from pairs where the male had been infected produced infection in offspring but not the reverse because no females became infected (Tables IV, V). Infection was not detected in the first litter when young were examined at 34-56 days of age. Infection was detected in the second and subsequent litters when young were examined at 35-61 days of age. Infections were first detected in litters exposed to experimentally infected male sires 44-81 DAI, i.e. in the second and subsequent litters. Prevalence of infection was high in litters sired by males from 44-133 DAI (47.9 %) (Table IV). Infection was not detected in offspring of a second litter which were < 35 days old however, when siblings where examined at ≥ 35 days, *M. borreli* were recovered from most littermates (Table IV). Thirteen male and 11 female mice from litters sired by infected males were infected with *M. borreli*.

A large amount of variability in the stage of development of *M. borreli* with host age was apparent in breeding experiments but in general, adults with unembryonated eggs were recovered from mice 35-46 days

Route of infection	Sex	Stage of nematode used			
		Au	Ae	Al	Lu
Subcutaneous					
	Male	2	10	5	9
	Female	2	7	4	5
Intraperitoneal					
	Male	2	4	4	8
	Female	2	5	4	8
Oral					
	Male			2	6
	Female			2	4
Percutaneous					
	Male				5
	Female				5

Au: adult with unembryonated eggs; Ae: adult with embryonated eggs; Al: adult with larvae; Lu: active larvae dissected from uterus.

Table II. – Numbers of BALB/c mice used to determine stage of *Muspicea borreli* and route of administration to achieve experimental infection of mice.

Direct transmission from male to female

Mouse No.	Time with female (DAI)	Total time with female (days)	Transmission to female
#1	0-137	137	yes
#2	0-145	145	yes
#3	0-149	149	yes
#4	25-31	6	no
#5	102-145	43	yes

Direct transmission from female to male

Mouse No.	Time with male (DAI)	Total time with male (days)	Transmission to male
#6	0-99	99	no
#7	0-112	112	yes
#8	0-137	137	no

DAI: days after inoculation of male or female with *Muspicea borreli*.

Table III. – Experimental infection of BALB/c mice with *Muspicea borreli* to test direct transmission from male to female and from female to male.

of age, adults with embryonating eggs were recovered from mice 46-53 days of age and adults with well developed and active larvae were recovered from mice 53-61 days of age. Transmission of infection occurred from experimentally infected males to their female partners on six occasions and the reverse on one occasion (Tables III, IV).

The delayed mating experiment resulted in litters of four, eight, nine, two and 10 young. *M. borreli* was found in three of the four males of the first litter examined at 37 days of age, in one female of the second litter examined at 37 days of age and in one male and three females examined at 54 days of age, but in none of the young of the third and subsequent litters. *M. bor-*

Mouse No.	Time adults with young (DAI)	Age of young at post mortem (days)	No. infected of No. examined	Adult post mortem (DAI)	Status of adults at post mortem
#9	21-42	37	0 of 4	58	M – ve, F – ve
#10	22-43	35	0 of 6		
#11	22-43	36	0 of 4		
#12	22-43	36	0 of 4	58	M – ve, F – ve
#13	24-44	35, 40, 53, 56	0 of 4		
#14	25-44	34, 39, 53, 56	0 of 4		
#15	28-50	56	0 of 7		
#16	30-50	38	0 of 7		
#17	31-52	36	0 of 5		
#10	43-64	39	0 of 4		
#11	44-66	**37, 40, 54, 61,** 61, 61	4 of 6	105	M + ve, F – ve
#13	45-64	38	0 of 4	83	M – ve, F – ve
#14	50-71	25, 29, **35, 35**	2 of 4	85	M + ve, F – ve
#15	51-71	**38, 38, 60, 60, 60, 61, 61,** 61	7 of 8		
#16	52-73	25, 28, **35**	1 of 3	87	M + ve, F – ve
#17	59-81	25, 28, **39, 39, 53**	3 of 5		
#10	81-102	25, **46, 46**	2 of 3	127	M + ve, F – ve
#15	89-109	38, 38, 38, 44, 44	0 of 5		
#17	95-116	26, 26, 26, **36, 53, 53,** 53	3 of 7		
#15	112-133	26, **53, 54**	2 of 3	166	M + ve, F + ve*
#17	126-148	59	0 of 10	185	M + ve, F + ve*

DAI: days after inoculation of male with *Muspicea borreli*; M – ve: male not infected; F – ve: female not infected; M+ve: male infected; F + ve: female infected.
Figures in bold in column 3 represent young infected with *Muspicea borreli.*
* Infection of female from male

Table IV. – Experimental infection of male BALB/c mice with *Muspicea borreli* to test direct transmission from male parent to offspring.

Mouse No.	Time adults with young (DAI)	Age of young at post mortem (days)	No. infected of No. examined	Adult post mortem (DAI)	Status of adults at post mortem
#18	22-44	44	0 of 3		
#19	23-45	44	0 of 7	57	F – ve, M – ve
#20	24-44	35, 35, 40, 54, 57, 57	0 of 6		
#21	24-44	42	0 of 3		
#22	35-56	37, 41, 41, 41, 45, 45, 46, 51, 53	0 of 9		
#23	35-56	42, 42, 45, 45, 50, 50	0 of 6	85	F – ve, M – ve
#18	44-66	45, 45, 50, 50, 52, 52	0 of 6		
#20	44-66	45, 45, 50, 50, 52, 52	0 of 6		
#21	46-66	39, 39, 46, 46	0 of 4		
#22	63-84	39, 39, 39, 43, 43, 43, 45, 45, 45, 52	0 of 10		
#18	66-87	35, 35, 47, 47, 49, 49, 49	0 of 7	115	F – ve, M – ve
#20	76-98	39, 39, 39, 43, 43, 43, 45, 45, 45, 52	0 of 10	128	F – ve, M – ve
#21	87-112	41, 41, 43, 45, 50	0 of 5	137	F – ve, M – ve
#22	109-130	38	0 of 8	147	F – ve, M – ve

DAI: days after inoculation of female with *Muspicea borreli*; M – ve: male not infected; F – ve: female not infected.

Table V. – Experimental infection of female BALB/c mice with *Muspicea borreli* to test direct transmission from female parent to offspring.

reli was not found in either parent examined 246 days after inoculation of the male.

Larvae of *M. borreli* survived and remained active for about an hour in balanced saline solutions (pH = 7.2) and in human saliva but died in tap water (pH = 7.1) and 1 % pepsin solution (pH = 2).

DISCUSSION

Prevalence of *M. borreli* in wild populations of *M. domesticus* was variable and relatively low. Infection in wild mice from southeastern New South Wales sites was observed exclusively in males; infection in wild mice from Canberra was observed in both sexes but with a higher prevalence in males (20.5 %) than females (9.4 %). Singleton (1985) reported that prevalence of *M. borreli* in 355 *M. domesticus* from the Australian Mallee wheatlands from November 1982 to November 1983 was 1.7 % with a mean intensity of 4.5 ± 2.00. This same author later reported prevalences ranging from < 2 % to > 60 % from 996 *M. domesticus* in the wheatlands from November 1982 through to April 1985 (Singleton, 1987). Peak prevalence corresponded with a period of high prevalence of other endoparasites. In general, this period of highest prevalence corresponded with a period of rapid population decline after plague densities had been reached and when the general condition of mice was relatively poor.

Experimental infection of BALB/c mice occurred only via subcutaneous inoculation but was achieved using *i)* adults containing embryonating eggs, *ii)* adults containing active larvae and *iii)* active larvae of approximately 0.5 mm in length dissected from the uterus of female worms. It proved extremely difficult throughout this study to experimentally infect female BALB/c mice with *M. borreli*. Data on prevalence and intensity of *M. borreli* infection in wild mice (Table I), the results of experimental infection of male and female BALB/c mice (Table III) and the results of the transmission studies among breeding pairs of BALB/c mice (Tables IV, V) prompt the conclusion that *M. borreli* is primarily a parasite of male mice. In marked contrast to this however, is the finding that there was no significant difference in the prevalence of infection in male and female BALB/c mice in litters sired by infected males. Singleton & Hay (1983) identified three social classes of wild male *Mus domesticus* (as *Mus musculus*) in enclosure studies on the effect of social organisation on reproductive success and gene flow in colonies in Australia. Alpha-dominant males initiated aggressive interactions and responded aggressively if confronted by other males. Subdominant males were aggressive initially but consistently retreated from agonistic encounters with the α-dominant males and kept to the periphery of defended areas. Subordinate males initiated few if any aggressive interactions and were submissive. Agonistic encounters were far less common between females than between males. All male bodies examined by them after death had wounding and scarring. Agonistic behaviour may be responsible for the pronounced male bias in prevalence of infection in the wild. Such behaviour was not observed in the BALB/c mice in the experimental transmission studies reported here and may have resulted in no significant difference in prevalence of infection between males and females sired by infected males.

Experimental infection of mice by subcutaneous inoculation of active larvae approximately 0.5 mm in length and dissected from the uterus of female *M. borreli* indicates that larvae develop to the infective stage in the uterus of adult worms and suggests that *i)* an obligate developmental phase on the host skin is unlikely and *ii)* autoinfection is possible.

When male mice were inoculated subcutaneously with *M. borreli*, immediately mated with uninoculated females and permitted to bred for 90 days infection was detected only in offspring of the second and subsequent litters or in their female partners. Transmission must have occurred within 21 days of birth because young were weaned at this time and housed separately from the breeding parents. *M. borreli* were found in offspring of the second and subsequent litters only after 35 or more days of age. When a male mouse was infected experimentally with *M. borreli* but not mated until 23 days later, transmission occurred to three of the four male siblings produced in the first litter and to one of the male and four of the female siblings produced in the second litter. Consequently, the life cycle of the parasite is direct and the pre-patent period in the mouse is estimated at 50-60 days.

Brumpt (1930) concluded that a free-living stage probably did not occur in *M. borreli* because wild mice placed in a cage with the old litter of a previously infected colony did not become infected. Bain & Chabaud (1974) observed that the musculature of *M. borreli* larvae was poorly developed in comparison with that of *Lukonema* spp. Consequently, the larvae of the latter might be able to survive in an external situation for a considerable period and be capable of migrating to the host while the larvae of the former would not. Brumpt (1930) concluded that mice probably ingest infective larvae crawling on the skin when they groom themselves, especially after bathing. The precise mode of natural transmission of the parasite in breeding pairs of laboratory mice was not determined and we were unable to establish infection in mice via an oral or percutaneous route. Nevertheless, larvae of *M.borreli* survived and remained active for about an hour in balanced saline solutions (pH = 7.2) and in human saliva but died under conditions emulating free-living (tap water pH =

7.1) and stomach (pepsin solution pH = 2) environments. Clearly, transmission was not effected by transplacental, transmammary or transseminal routes and we have demonstrated, as did Brumpt (1930), that an intermediate host is not involved in transmission. Thus, it is difficult to conclude other than as did Brumpt (1930), that transmission may occur via penetration of skin or mucous membranes and we believe that allogrooming behaviour, close contact and nest sharing may be particularly important in this regard.

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